Recombinant Protein Therapeutics from CHO Cells — 20 Years and Counting

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The CHO cell is at its height of technological prominence thanks to its adaptability to various culture conditions and plasticity in the context of genetic alterations. With further research, the application of cell culture strategies based on scientific reasoning, rather than heuristics, is in the not-so-distant future.

Recombinant protein therapeutics have changed the face of modern medicine in the past decade, and they continue to provide innovative and effective therapies for numerous previously refractory illnesses. Today, they are used in the treatment of a variety of human diseases, ranging from cancers to infertility. These proteins are generally synthesized by large-scale cultivation of genetically engineered "host" cells, which harbor artificially transfected genes encoding for the proteins of interest. For protein therapeutics to be effective, they must be synthesized in biologically active forms, requiring proper folding and post-translational modifications. In many cases, this includes glycosylation, a type of modification where certain carbohydrate moieties are added to specific amino acid residues of the protein.

Glycoproteins, as these are usually called, are generally synthesized in mammalian cells, because common microbial hosts like *Escherichia coli* lack the requisite machinery to synthesize appropriate glycoforms. Several rodent- or human-derived cells like 3T3, CHO, BHK, HeLa and HepG2 are frequently used in biomedical research for heterologous protein expression. Despite the availability of a plenitude of cell lines, nearly 70% of all recombinant protein therapeutics produced today are made in Chinese Hamster Ovary (CHO) cells. The current annual sales for biologics produced using CHO cells alone exceed US$30 billion worldwide.

The first recombinant therapeutic protein produced in mammalian cells, tissue plasminogen activator (r-tPA, Activase) synthesized using CHO cells, was approved for clinical use in 1987. This marked the beginning of an array of highly successful CHO-based therapeutics (Table), which continue to revolutionize the field of medicine to this day. The knowledge and expertise amassed over the past two decades will most certainly ensure that CHO cells continue to remain the industry's premier workhorse for therapeutic protein production, at least in the near future.

In this article, we revisit the evolution of CHO cells, tracing from their origins to the development of current production cell lines, and discuss some important aspects that make them robust and versatile protein expression hosts. Unlike some of its rodent relatives (mouse and rat), the generation of genomic resources for Chinese hamster has been quite limited. Genomic and proteomic tools using these resources can potentially aid in understanding and improving recombinant protein production processes leading to a significant enhancement in the speed with which these therapeutics transition from laboratory molecules to life-saving medicines. We, therefore, devote special attention to the value and need for such information in meeting the challenges of future research and manufacturing processes.

From hamster to tissue cultures

Chinese hamsters were first used as laboratory specimen in 1919 in place of mice for typing pneumococci (Box). Subsequent efforts at domestication by Dr. George Yerganian and others in the mid-20th century led to the development of spontaneous hereditary diseases due to inbreeding, spurring research interest in hamster genetics (*1*, *2*). It was noted during that time

Table. Selected list of approved biologics produced in Chinese Hamster Ovary cell lines.

Product	Type	Therapeutic use	Manufacturer	Year of approval (FDA)
Vectibix	Anti-EGFR mAb	Metastatic colorectal cancer	Amgen	2006
Myozyme	α-glucosidase	Pompe disease	Genzyme	2006
Aldurazyme	Laronidase	Mucopolysaccharidosis I	Genzyme	2006
Orencia	Ig-CTLA4 fusion	Rheumatoid arthritis	Bristol-Myers Squibb	2005
Naglazyme	N-acetylgalactosamine-4-sulfatase	Mucopolysaccharidosis VI	BioMarin Pharmaceutical	2005
Luveris	Luteinizing hormone	Infertility	Serono	2004
Avastin	Anti-VEGF mAb	Metastatic colorectal cancer & lung cancer	Genentech	2004
Advate	Factor VIII (engineered)	Hemophilia A	Baxter	2003
Xolair	Anti-IgE mAb	Moderate/severe asthma	Genentech	2003
Raptiva	Anti-CD11a mAb	Chronic psoriasis	Genentech	2003
Fabrazyme	α-galactosidase	Fabry disease	Genzyme	2003
Rebif	Interferon-β	Relapsing multiple sclerosis	Serono	2002
Humira	Anti-TNFα mAb	Rheumatoid arthritis	Abbott	2002
Aranesp	Erythropoietin (engineered)	Anemia	Amgen	2001
Campath	Anti-CD52 mAb	Chronic lymphocytic leukemia	Genzyme, Bayer	2001
ReFacto	Factor VIII	Hemophilia A	Wyeth	2000
Tenecteplase	Tissue plasminogen activator (engineered)	Myocardial infraction	Genentech	2000
Herceptin	Anti-HER2 mAb	Metastatic breast cancer	Genentech	1998
Enbrel	TNFα receptor fusion	Rheumatoid arthritis	Amgen, Wyeth	1998
Benefix	Factor IX	Hemophilia B	Wyeth	1997
Follistim/Gonal-F	Follicle stimulating hormone	Infertility	Serono/NV Organon	1997
Rituxan	Anti-CD20 mAb	Non-Hodgkin's lymphoma	Genentech, Biogen Idec	1997
Avonex	Interferon-β	Relapsing multiple sclerosis	Biogen Idec	1996
Cerezyme	β-glucocerebrosidase	Gaucher's disease	Genzyme	1994
Pulmozyme	Deoxyribonuclease I	Cystic fibrosis	Genentech	1993
Epogen/Procrit	Erythropoietin	Anemia	Amgen/Ortho Biotech	1989
Activase	Tissue plasminogen activator	Acute myocardial infraction	Genentech	1987

that the low chromosome number of Chinese hamsters (2n = 22) made them particularly useful models in radiation cytogenetics and tissue culture studies. In 1957, while investigating the usefulness of various cells in somatic cell genetics, Dr. Theodore T. Puck of the Dept. of Medicine at the University of Colorado isolated an ovary from a female Chinese hamster and established the cells in culture plates (*3*). It soon became obvious that these cells were quite resilient and lent themselves readily to *in vitro* cultivation with relatively fast generation times. Karyotype heterogeneity among these cell populations evoked particular interest in the context of studying chromosomal abnormalities.

Contribution to basic biomedical research

Until the later part of 20th century, isolation and characterization of mammalian cell mutants for cytogenetic studies was a challenging exercise, fraught with failures because, unlike microbes, mammalian cells are generally diploid. The establishment of CHO cells in tissue cultures enabled researchers to overcome this difficulty because these cells were functionally hemizygous for many genes, primarily due to gene inactivation (*4*, *5*). CHO cells have, thereafter, been used in numerous biomedical studies ranging from analysis of intermediary metabolisms and cell cycle to toxicology studies, so much so, that they have been termed as the mammalian equivalent of the model bacterium, *E. coli* (*6*).

Among the historically important medical and cell biology studies conducted in CHO, it was the early work

A Brief History of the Chinese Hamster

Chinese hamsters (scientific name, *Cricetulus griseus*) belong to a family of rodents that are native to the deserts of northern China and Mongolia. They have been used in life-saving biomedical research ever since they were first introduced into the laboratory in 1919 for typing pneumococci. In the early 1920's, they gained reputation as valuable tools in epidemiological research, because they were known as carriers of the deadly parasite *Leishmania* causing kala-azar (also known as black fever or leishmaniasis). In 1948, they were literally smuggled into the U.S. by Dr. C. H. Hu and Dr. Robert Watson, who were later accused for 'war crimes' by the Germ Warfare Commission of China, leading to imprisonment of the former (*1*). Amid the turmoil between China and the U.S. in the early cold war days, it was thought that the U.S. would use them as agents of biological warfare by infecting them with deadly diseases like cholera or plague and parachuting them over Manchuria. Instead, Chinese hamsters are now credited with saving thousands of lives from illnesses like cancer every year.

involving mutagenesis of these cells and isolation of certain auxotrophs (*7*) that facilitated their migration from laboratory benches to industrial reactors. These mutants exhibited particular nutritional requirements for maintaining growth and viability over long culture periods. Mutants with varying degrees of deficiencies in metabolic enzymes like adenine phosphoribosyl transferase (APRT) and dihydrofolate reductase (DHFR) were isolated in this manner (*8*, *9*). In addition, mutants defective in transcription, translation and machineries for certain amino acid and polyamine biosynthesis were also isolated (*10–14*).

Whilst the primary motive behind the isolation of these mutants was fundamental research, it was, perhaps, fortuitous that the nutritional requirements of auxotrophs could be put to use for selection of cells expressing exogenous proteins. The DHFR expression system, which will be discussed in later sections, is now a standard tool for molecular biologists requiring vector-mediated gene transfer into CHO cells. This ability to transfect, select, amplify, and stably express biologically active heterologous proteins soon became an immense boon for biopharmaceutical companies involved in the business of large-scale protein therapeutic synthesis.

The immense adaptive ability of CHO cells and their ease of maintenance have been exploited in many fields of basic biomedical research. G-protein coupled receptors and their associated signaling pathways are a class of molecules commonly studied by stable expression in CHO cells, making contributions to patient treatment in the fields of anesthesiology, pharmacology and basic cellular signaling research as well (*15–17*). The anchorage-dependent characteristics of CHO cells make them useful models for understanding cytoskeletal and microtubule structure, adhesion, and motility, contributing to knowledge in proliferation control, cancer biology and treatment (*18*, *19*). CHO cells are also relatively easy to synchronize, making them ideal models for cell cycle studies (*20*).

DNA damage and repair is another field where CHO cells are used extensively. Mutants deficient in double-stranded DNA break repair have been isolated and studied in DNA repair and radiation research (*21*, *22*). CHO cells are also used in other toxicology and drug addiction studies, and are readily amenable for high-throughput biomolecular screening systems. Some recent studies in this context have involved the analysis of the effects of caffeine (*23*), pesticides (*24*), and various therapies used in cancer treatment (*25*) on mammalian cells.

CHO cells as recombinant DNA hosts

The choice of host cells for protein expression has a profound impact on product characteristics and maximum attainable yields. Protein folding and post-translational modifications conferred by the hosts dictate the phamacokinetic and pharmacodynamic properties of the products, and hence their solubility, stability, biological activity and residence time in humans. Product safety is another key aspect that must be considered in choosing host cells. The production host must not allow the propagation of any adventitious pathogenic agents that may eventually find their way into humans. From an industrial perspective, the ability to adapt and grow cells in suspension instead of adherent cultures is highly desirable as it allows volumetric scalability and use of large stirred-tank bioreactors. Finally, the host cells must be amenable to genetic modifications allowing easy introduction of foreign DNA and expression of large amounts of desired protein.

Twenty years of experience with CHO cells in the biopharmaceutical industry has demonstrated that, to a large extent, they possess many of these characteristics. CHO cells have a proven track record for producing proteins with glycoforms that are both compatible and bioactive in humans. They have also been demonstrated as safe hosts for synthesis of biologics. One of the early concerns in recombinant protein production was that cultured mammalian cells were presumably derived through perturbation of oncogenes, and thus, can proliferate without the effects of senescence. However, CHO cells have been proven safe, with the value of products being generated considerably outweighing any associated risks.

Downstream processes for CHO cell products have matured to a stage where they can be purified to contain not more than picogram levels of contaminating CHO DNA per dose of the product (*26*). Also, a study in 1989 found that, of 44 human pathogenic viruses tested, the vast majority of them including HIV, influenza, polio, herpes and measles do not replicate in CHO (*27*). From a regulatory standpoint, CHO cells have withstood the test of time. Extensive testing and safety data accumulated during two decades of commercial production will doubtless ease the process of achieving FDA approval for production of new CHO-based therapeutics.

Yet, arguably, the most important factors that enabled the adoption of CHO cells as the industry's workhorse are their adaptability and their ease of genetic manipulation. CHO cells are quite adaptable and can grow to very high densities in suspension cultures that are readily scaled to >10,000-L bioreactors. Also, the isolation of cells deficient in DHFR enzyme has led to an effective means for the selection of stable clones and amplification of genes, thereby dramatically increasing specific productivity levels.

DHFR is a small monomeric enzyme that catalyzes the conversion of folic acid, a common vitamin, to tetrahydrofolate (THF). The latter is a cofactor carrier for one-carbon moieties required in various biosynthetic reactions, particularly synthesis of glycine, purine and thymidine. In a pioneering work during the early 1980s,

Chasin and co-workers isolated two DHFR-deficient mutants (DXB11 and DG44) that are commonly used as parental cell lines today (*9*, *28*, *29*). Both these are derivatives of a proline requiring auxotroph established by Kao and Puck in 1968 (*30*).

Since DHFR-deficient cells are triple auxotrophs for glycine, hypoxanthine (a purine derivative) and thymidine, introduction of heterologous genes into cells can be accomplished by co-transfection with a functional copy of the DHFR gene, which obviates the need for these nutrients. Clonal selection is then performed by growth in media devoid of glycine, hypoxanthine and thymidine. The DHFR system also enables efficient amplification of the cloned DNA. When cultured in high levels of methotrexate (MTX), a folic acid analog that blocks DHFR activity, transfected cells must cope with the decrease in DHFR activity. The mere presence of an exogenous DHFR gene is insufficient for survival under these conditions; rather, an increased expression of DHFR is warranted and surviving cells have likely amplified the copy number of DHFR locus to accomplish this. The genetic linkage between DHFR and the transfected gene of interest then ensures that the transgene is also co-amplified, thus enhancing chances of securing a high producing strain.

Current strategy for CHO cell line development

The commonly employed strategy for deriving a high-producing CHO cell line from parental lines using the DHFR selection system, although well established, is quite time-consuming and laborious. Figure 1 is a representative illustration of typical steps involved in development of a producing cell-line. It is not uncommon for this process to take more than six months in industrial settings for each new candidate therapeutic even before it can enter the evaluation phase when the molecule is tested for its efficacy and safety in animal/human subjects.

The first step in developing a producing cell line is delivery of the recombinant DNA into the host cell nucleus for chromosomal integration (Step 1). Several methods, like calcium phosphate precipitation, electroporation, lipofection and retroviral transfection, are commonly used with optimized protocols available in literature. Once the DNA enters the nucleus, the integration site of the vector is random, and expression of the transgene is, in part, dictated by the surrounding chromosomal structure and associated features. High transcriptional activity is extremely desirable at the locus of DNA integration. Various strategies have been attempted to improve the frequency of isolation of clones that have genes integrated at transcriptionally active sites. For example, a mutant DHFR gene with reduced enzymatic activity or a DHFR gene driven by a weak promoter is sometimes used as a

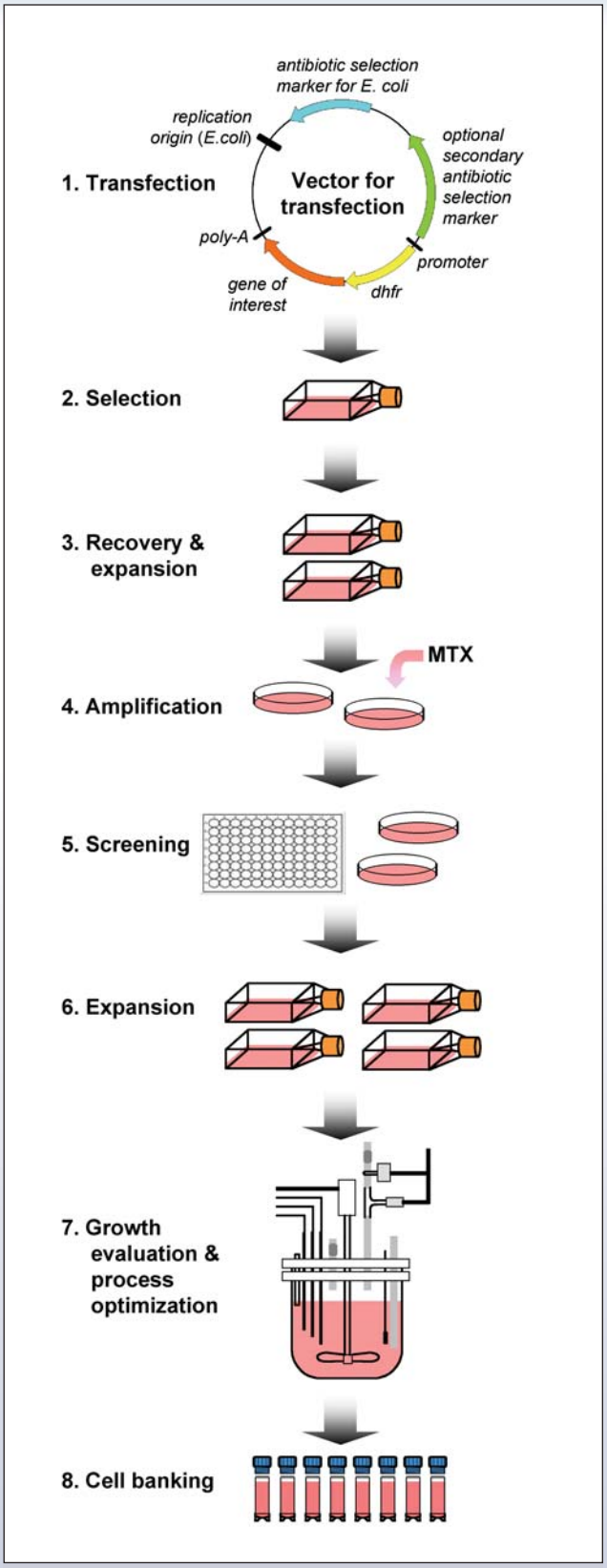

Figure 1. A schematic of steps involved in cell line development for production of recombinant proteins using CHO cells.

more stringent selection leading to higher transgene expression. Other strategies include use of chromatin opening elements like scaffold or matrix attachment regions (S/MARs) (*31*) and ubiquitous chromatin opening elements (UCOS) (*32*) in the vector DNA, which promote accessibility of DNA for transcription at the integration site.

Following DNA delivery, a pool of cells stably expressing the co-transfected DHFR enzyme are selected using low levels of MTX and cultivation in the absence of glycine, hypoxanthine and thymidine (Step 2). Additional selection agents like antibiotics (*e.g.*, hygromycin) may be used when appropriate resistance markers are included in the vector. At this stage, the majority of cells that did not successfully integrate the vector DNA are killed, and surviving cells, presumably expressing the protein of interest, are eventually recovered (Step 3). Next, this pool of cells is exposed to high concentrations of MTX (typically ramped over 0.5-1 μM). This step, commonly referred to as amplification (Step 4), dramatically increases the selection pressure. In order to survive, CHO cells typically undergo genomic rearrangements and amplification of the locus of DNA integration resulting in increased copy numbers for both DHFR and the protein of interest. Often, clones containing several hundred copies of the vector construct can be found following amplification.

In most cases, this step leads to the isolation of a pool of cells enriched for clones with a high specific productivity. This pool, however, is heterogeneous, containing cells with different integration sites, copy numbers and varying specific productivities. Individual clones with the highest possible productivity and growth rates, and the best product quality need to be isolated. This is accomplished by a series of limiting dilutions in multi-well plates (Step 5), so that single colonies having uniform cell populations can be isolated. Typically, a large number (few hundreds) are screened to isolate a small number (~10–30) of candidate production cell lines.

The chosen clones are expanded through several rounds of passaging (Step 6) and each clone is evaluated in lab-scale bioreactors under conditions mimicking those encountered in large-scale production facilities (Step 7). After evaluation of key parameters, a single production cell line is chosen and banked as frozen vials for future use (Step 8). This cell line is subsequently used for generating clinical trial materials and, if successful, eventually employed in commercial manufacturing.

Accomplishments of cell culture technology

One of the major challenges in using CHO and other mammalian cell lines as recombinant protein production hosts is that the volumetric yields of protein produced from processes using these cells are relatively low. The productivity of mammalian cell culture processes is typically ~10–100-fold lower than what can be achieved using microbial host systems. This necessitates the construction and maintenance of very large and costly production facilities. Over the past two decades, the biopharmaceutical industry has largely been able to meet the growing demand for mammalian cell based therapeutics through selection of better production cell lines and optimization of culture strategies for higher yields, as opposed to linear expansion of culture volumes.

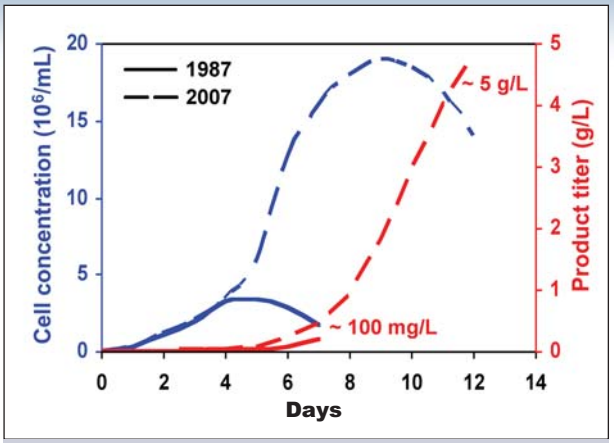

Figure 2. Typical growth and production curves illustrating cell line and process improvements accomplished in the past 20 years.

Figure 2 shows the significant improvements made in mammalian cell culture technology over the past two decades. Many of the first processes were run in simple batch modes, lasting about seven days. Cell concentrations typically peaked at $3x10^6$ cells/mL and final titers of ~100 mg/L were considered reasonable. Today, most processes employ fedbatch cultures, which allow for higher cell density and longer culture durations (typically 10–12 days) by addition of concentrated medium throughout the culture to replenish depleted nutrients. Improvements in screening and cell line development, as well as medium optimization and process monitoring and control have also contributed to the enhanced specific productivity levels achieved today. Currently, final product titers of 1–5 g/L are a routinely realized.

Challenges ahead

Despite significant advances, the current paradigms of cell line development remain, to a large extent, empirical. There is a considerable degree of variability and very little understanding of the sources of variation in mammalian cell culture processes. In the absence of any significant comprehension of the underlying cytogenetic events accompanying cell line development, laborious and extensive screening of clones, often spanning over several months, is still widely practiced in industry.

Thus, each new candidate therapeutic needs a dedicated team of scientists for development, characterization

and optimization of an essentially new process. This is especially problematic during scale-up because there are no reliable methods for predicting or modeling the growth characteristics and production capabilities of cell clones in large-scale bioreactors. The clones selected and characterized in bench-top reactors may not behave in similar ways in large-scale bioreactors, despite employing seemingly identical process parameters.

These difficulties primarily stem from our inadequate understanding of the biology and physiology of mammalian cells. The next phase of development should, therefore, seek to investigate the underlying mechanisms leading to generation of clones producing large amounts of good quality products. Important clues have already emerged and several strategies have been employed to engineer cells with enhance productivity (*33*).

Cell viability can be significantly enhanced by limiting lactate production levels in culture. Efforts have been made to engineer energy metabolism pathways in CHO to achieve this effect (*34–36*). Other efforts have sought to understand cell cycle mechanisms and identify potential triggers for apoptosis and cell death (*37*, *38*). Since heterologous protein production places abnormal stress on host cells, some studies have focused on the effects of large-scale protein production on host cell machinery (unfolded protein response). Lipid metabolism and membrane biogenesis is yet another area that has evoked interest because membrane turnover rate in protein secreting cells is likely to be rather high (*39*). For enhancing product quality, some studies have employed engineering of glycosylation enzymes, with the case of erythropoietin being well documented (*40*).

The success of these approaches can be significantly enhanced by harnessing the power of genomics- and proteomics-based technologies. These technologies are primarily based on DNA microarray and mass spectrometry platforms and provide a glimpse into the molecular machineries modulating cellular responses to genetic and environmental manipulations. With these tools, a researcher can profile the expression levels of thousands of genes in one experiment, and compare a number of conditions encountered during cell line development. By using these tools, researchers have identified key apoptosis genes involved in the control of CHO cell viability during fedbatch cultures (*41*).

Cellular changes associated with increased productivity in CHO cells have been probed using 2-D gel electrophoresis and mass spectrometry (*42*). The effects of low temperature on proteome profile and protein productivity have been analyzed in another study (*43*). These and other similar studies should provide future researchers with potential gene targets that can be manipulated to enhance productivity and cell growth. Bioinformatic tools for analyzing the data generated from these experiments, to identify gene expression patterns and gene-trait relationships, are rapidly maturing.

The CHO genome project

The landmark completion of the human genome project in 2001 has accelerated the discovery of human disease markers and drug targets for treatment of many chronic ailments ranging from cancers to Alzheimer's disease. Subsequent sequencing of other mammalian genomes, like mouse, dog and rat, which are common laboratory models, has further enabled scientists to perform biomedical research and genetic analyses using these organisms with much greater efficiency. While many other organisms of medical, ecological, and agricultural importance are now in line to be completely sequenced (www.genome.gov/10002154), it is somewhat surprising that the Chinese hamster is not among them.

The success stories of the mouse, rat and human genomes are a compelling motivation to undertake a similar effort for the Chinese hamster. The promises of molecular tools, such as microarrays, high-throughput proteomics, quantitative real-time PCR and RNA interference, can be realized to the fullest extent, only when the complete genome sequence or, at least, the gene-coding and regulatory element sequences of the organism are available. Through a collaborative effort between the University of Minnesota and the Bioprocessing Technology Institute of Singapore (A*STAR), an effort to bring genomics, proteomics, and other -omic based technologies to cell culture engineering was initiated in 2002. A cDNA-based microarray with more than 4,000 different CHO sequences was created in 2004 (*44*). The initial work convinced the researchers that Chinese hamster was sufficiently distinct from mouse and rat, so that the genomic tools developed for those rodents are not directly applicable for probing gene expression changes in Chinese hamster using cross-species analysis techniques. This spurred an effort to seek more comprehensive resources and expand sequencing efforts. In partnership with the Society for Biological Engineers (SBE), the Consortium on Chinese Hamster Ovary Cell Genomics was founded in 2006. Currently, eight corporate partners from pharmaceutical and biotech industries form the core of this consortium and the number is growing. In the one and a half year since its inception, the consortium has greatly accelerated the development CHO genomic resources.

Altogether, over 80,000 ESTs (expressed sequence tags, essentially RNAs with polyA tails) have been sequenced and assembled into over 27,000 unique non-overlapping sequences, and have been used to create both Affymetrix and cDNA microarray platforms for transcriptome profiling. Continued sequencing efforts using both traditional

and new sequencing technologies are focusing on generating more open reading frame sequence for translated proteins, as well as upstream regulatory regions and the increasingly important "non-coding" regions of the genome (*e.g.*, microRNAs). The sequences have been annotated based on their homology to other rodent or human sequences (using databases like GenBank, Ensembl, FANTOM etc.). This enables researchers to tap into the vast resources of mouse, rat and human data to better analyze gene expression changes in CHO.

For example, by mapping CHO ESTs to well annotated genomes (*e.g.*, mouse, human, and rat) the transcriptome profile of CHO cells under different culture conditions can be visualized on metabolic maps created for other species. In addition, chromosome mappings to mouse, human, rat and dog have also been performed (*45*). During evolution, genomic sequences are rearranged, but not entirely in a random fashion; rather, large chunks of chromosomes are relocated together, forming new chromosomes by re-assortment of these segments. Within each large segment, the order of genes is largely preserved. By knowing which regions are conserved between Chinese hamster and mouse (*i.e.*, have "synteny"), one can make educated guesses regarding the structure and content of unsequenced regions lying between any two adjacent sequenced loci. Figure 3 shows the regions of conservation between Chinese hamster and mouse chromosomes identified based on EST sequencing and whole genome alignment.

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The past few years have seen an increased use of proteomics and genomics tools for cell culture bioprocessing research. Both DNA microarrays and quantitative proteomic tools, such 2D gel electrophoresis and iTRAQ, have been used to explore genes associated with enhanced productivity. The CHO DNA microarray created by the consortium has begun to afford its members the ability to apply transcriptome analysis to various CHO producing lines. The question of paramount importance is: "Can we identify the genetic markers associated with high productivity?" In a related context, it will be interesting to analyze how CHO cells, which do not secrete any significant amounts of proteins naturally, gained extraordinary secretory capabilities rivaling that of professional secretors in our body like plasma or liver cells.

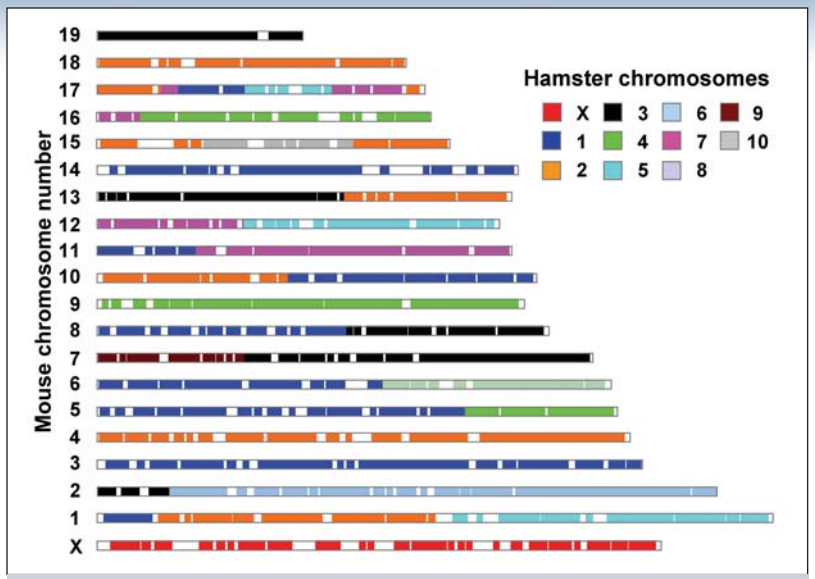

Figure 3. Regions of conserved synteny between Chinese hamster and mouse chromosomes.

Another potential application of microarrays is the analysis of process variability. Transcriptome analysis using DNA microarrays is arguably the most readily available -omic tool. No other technique can be applied so quickly to comprehensively survey the levels of numerous variables. While generation of such data is fairly routine, systematic tools that can directly link transcriptome data with physiology are still in their infancy. Nevertheless, transcriptome analysis can establish process fingerprints for manufacturing runs. Manufacturing runs are ideally repetitions of the "same" process every time in industry; however, in reality, these apparently "same" processes seldom provide identical product qualities and product titers. When process monitoring schemes fail to identify the cause of this deviation, changes in transcriptome profiles can provide diagnostic information. Once a sufficiently large amount of data accumulates, processes can be classified according to some criteria (*e.g.*, final productivity) and comparison of transcriptome data from these process classes may identify molecular signatures defining each class. This can lead to determination of critical parameters and enable one to devise measures for minimizing process variability.

Concluding remarks

Half a century after its establishment as a cell line and two decades after it brought the first mammalian recombinant therapeutic protein to clinical application, the CHO cell is at its height of economical and technological prominence that none could have imagined. Its remarkable adaptability to various culture conditions and immense plasticity in the context of genetic alterations contribute to its versatility. Despite its importance, the exact genetic basis for this versatility remains poorly understood. With a concerted effort, genomic resources for CHO cells are being expanded. These genomic resources, combined with systems biology approaches are making significant contributions to our understanding of the genetic basis of the complex traits of high productivity and high product quality. This will undoubtedly accelerate product and process development procedures. One can envision the application of cell culture strategies, in the not so distant future, where development of new therapeutics and processes will be guided largely by the principles of scientific reasoning, rather than heuristics. CEP

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